

HISTOPHYSIOLOGICAL STUDIES ON THE CORPUS ALLATUM OF *LEUCOPHAEA MADERAE*. I. NORMAL LIFE CYCLE IN MALE AND FEMALE ADULTS¹

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In the course of previous work in our laboratory dealing with the functional and cytological properties of neuroendocrine systems, in particular the inter-cerebralis-cardiacum-allatum system of the insect, *Leucophaea maderae* (Scharrer, 1946b; Scharrer and Scharrer, 1944), it was observed that the corpora allata display an impressive variability in their morphology. The question arose whether differences in the size and histological appearance of these glands reflect differences in age, sex, functional state, etc., as is the case in an analogous organ, the anterior pituitary, or merely constitute the range of individual variation, or perhaps both.

The first indication that physiological state determines structure in the corpus allatum of *Leucophaea* was obtained by an analysis of the results of experiments in which nerves connecting this organ with the brain were severed. Following this operation, when performed at the appropriate time, the functional capacity of the gland was stepped up as demonstrated in last instar nymphs (Scharrer, 1946a), and there was a marked increase in glandular volume and relative cytoplasmic content (Scharrer, 1952). The conclusion seemed justified that, at least under the conditions of these experiments, a large gland in which the nuclei are loosely distributed represents a physiologically active gland.

This conclusion was borne out further by more recent studies (Engelmann, 1957) describing cyclic changes in the size of the corpora allata of normal adult females of the same species, *Leucophaea maderae*, in conjunction with reproductive processes.

Another investigation in which corpus allatum volume could be correlated with functional change concerns the effect of gonadectomy (von Harnack and Scharrer, 1956). These observations gave rise to two questions: (1) Are the increase in the size of the corpus allatum following gonadectomy and that occurring after nerve severance unrelated though comparable phenomena, or is there a mechanism involved which operates in both instances, resulting in a similar histophysiological response? (2) Is the volumetric increase observed in the corpora allata after both types of operation equivalent to that occurring under normal physiological conditions, *i.e.*, merely a sign of "activation," or does the response of the operated specimens go beyond the normal physiological range characteristic of the corpora allata?

In order to answer these questions data are needed, in addition to those reported by Engelmann (1957), concerning variations in the morphology of the corpora

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allata in conjunction with physiological states throughout the adult life span of both males and females. Therefore, prior to reports on experimental results, the present paper deals with cytological evidence of secretory activity and variations in cell size, cell number, and nuclear-cytoplasmic ratio in the normal animal to the extent to which they can be examined with conventional techniques of light microscopy. These observations will serve as points of reference for subsequent papers which will deal with experimental observations.

MATERIAL AND METHODS

The material on which this investigation is based consists of 88 female and 46 male normal animals of known adult age. They were removed from stock colonies on the day of their emergence and isolated in pairs in pint-size jars. They were kept at room temperature on a routine diet of dog chow and fresh apple until the day of fixation. The animals were killed at selected intervals ranging from 0 to 599 days in the female, and from 0 to 471 days in the male series. In the majority of cases an autopsy was performed to determine the condition of the internal organs, particularly that of the reproductive organs of the females.

For the histological study of the corpora allata and associated endocrine organs, the heads were fixed in Zenker-formol. Among a variety of staining techniques tested the aldehyde fuchsin method (Gomori, 1950) proved most useful, when modified according to Halmi (1952) and Dawson (1953). The addition of Weigert's hematoxylin as a nuclear stain permitted the use of the sections for nuclear counts as well as a study of secretory products. Most of the tissues were cut serially at 7 μ , a small number of cases at 5 μ .

As a basis for comparison of the morphological characteristics of the corpora allata, three values were determined: the volume of both glands, the total number of nuclei, and their relative number per unit of tissue (nuclear-cytoplasmic ratio). In addition, nuclear size was measured in representative cases of large and small glands. Since it was considered desirable to include a large number of animals in the normal, as well as the experimental series which will be treated in subsequent papers, it became necessary to select methods of determining these quantitative values which afford a sufficient degree of accuracy without excessive expenditure of time.

(a) Determination of volume

The conventional method of estimating organ volume by measurements of each consecutive section in a series, when carried out in hundreds of specimens would be a staggering task. Therefore, methods based on the measurement of representative sections were explored for their validity in expressing organ volume. This seemed feasible because in our studies the aim was not so much to determine glandular volume as accurately as possible, but to select a convenient and reasonably valid numerical expression of quantitative differences. An additional advantage of such a simplified procedure is the possibility of using incomplete series of histological slides, a not infrequent result of the technical difficulties encountered with chitinous material.

The validity of the method to be adopted was tested as follows. To serve as a basis for comparison, the volumes of the corpora allata of 20 representative cases

were calculated in μ^3 as accurately as possible by drawing each consecutive section with the aid of a camera lucida and measuring the drawings with a planimeter. By the inclusion of the extremes in this group of cases the range of variability was accurately established. Next, the same 20 cases were evaluated by measuring only intermittent sections. A comparison of results showed inaccuracies to be still rather small when only every ninth section was measured. The average number of sections per corpus allatum being 40 to 50, approximately 6 measurements were available for the calculation of the volume of each gland. Estimated values obtained by calculating the means between each of two consecutive measured sections were substituted for the values of the "skipped" sections. The inaccuracy of this procedure when compared with the results of measuring every section turned out to be small enough to permit the adoption of the abbreviated method for this and the following papers of this series.

(b) Nuclear counts

An accurate determination of the density of nuclear distribution meets with certain difficulties especially in small corpora allata poor in cytoplasm. In addition to the crowding of nuclei, a certain degree of irregularity in nuclear distribution has to be taken into consideration. Different methods of estimation were tested; the most reliable figures were obtained by direct counts under the microscope with the aid of a micrometer disc added to the eyepiece and of a tally counter. In each corpus allatum three representative sections were selected in approximately the same location, *i.e.*, the largest section in the middle of the series plus the fifth section from the middle section on either side. The total area of the three sections representing each corpus allatum and the sum of nuclei counted in this area permitted the calculation of the number of nuclei per mm^2 , a figure which represents a fairly good index of nuclear density. These values are recorded in Figures 1 and 2.

In order to determine whether a volumetric increase in the corpora allata is due solely to a rise in cytoplasmic content or whether this is accompanied by an increase in nuclear number, an at least rough calculation of the absolute number of nuclei present in each pair of corpora allata studied became desirable. For this the following formula suggested by Engelmann (1957) was used:

$$N = \frac{N' \times V}{A \times (T + 2r)}$$

where N = total number of nuclei per pair of corpora allata, N' = number of nuclei counted in A (total area of three sections selected for nuclear counts), V = volume of both corpora allata, T = thickness of sections, $2r$ = average nuclear diameter.

RESULTS

a. Females

The aim of this study was to analyze in detail the periodic changes in the corpora allata in the course of one reproductive cycle, and to determine whether essentially the same pattern obtains in successive cycles throughout the adult life span. It soon became apparent (and was subsequently substantiated in our

TABLE I

Quantitative changes in the corpora allata of 26 females of Leucophaea maderae of varying adult age (maximum 599 days) grouped according to different stages in the reproductive cycle

	Number of animals	Range in volume of corpora allata (in mill. μ^3)	Range in number of nuclei	Range in nuclear-cytoplasmic ratio (nuclei/mm. ²)
Group A (small eggs in ovary; beginning of cycle)	8	5.0-10.8	3657-6411	733-2034
Group B (growing eggs in ovary)	3	9.0-12.4	4137-9488	864-1558
Group C (pre-ovulatory stage)	3	15.3-37.0	7017-15,591	825-987
Group D (pregnancy)	12	3.9-17.3	3124-11,361	1155-1978

experimental work) that the corpus allatum of *Leucophaea* shows a fair degree of structural variability within groups of specimens selected under comparable physiological conditions. This fact had to be taken into account in the search for significant correlations with functional states and necessitated the use of larger samples than would otherwise be called for.

There is also a certain degree of individual variation in the timing of the alternating periods of ovarian activity and quiescence characteristic of the reproductive activity of *Leucophaea*. Therefore, an analysis of the first cycle which begins after the emergence of the adult offers advantages because of the greater ease of dating. For the study of subsequent cycles the "adult age," *i.e.*, the time elapsed since emergence, is less significant than the conditions of the ovary at the time of fixation. Consequently the selection of appropriate stages in these later cycles is facilitated by the recording of preceding parturitions. This is taken into account in the arrangement of the data summarized in Figure 1. These include 62 females killed at intervals of a few days during the first and second reproductive cycles. The cases illustrating the first cycle are arranged according to adult age (days elapsed between emergence and fixation of the animal), those of the second cycle are grouped in reference to the interval between preceding (*i.e.*, first) parturition and fixation.

An additional 26 animals studied in this series encompass the remainder of the entire adult life span. The oldest specimens represent extremes in longevity obtained from a large collection of dated females and are of particular value in the search for possible changes in corpus allatum function with increasing age. This group of older females, dated according to adult age, does not lend itself for the same graphic representation as the younger specimens, since the number of reproductive cycles they had completed was not recorded in all cases. This group is, therefore, not included in Figure 1. The values obtained for these animals grouped according to phases of the reproductive cycle are summarized in Table I.

As can be seen from Figure 1, within 30 days after emergence the volume of the corpora allata rose from an average value of 4.2 million μ^3 (minimum 3.5) in the very young female to an average value of 15.3 million μ^3 , (maximum 16.6) corresponding to the time when the largest oocytes had almost reached their maximal size.

After ovulation, the corpora allata returned to approximately the same size

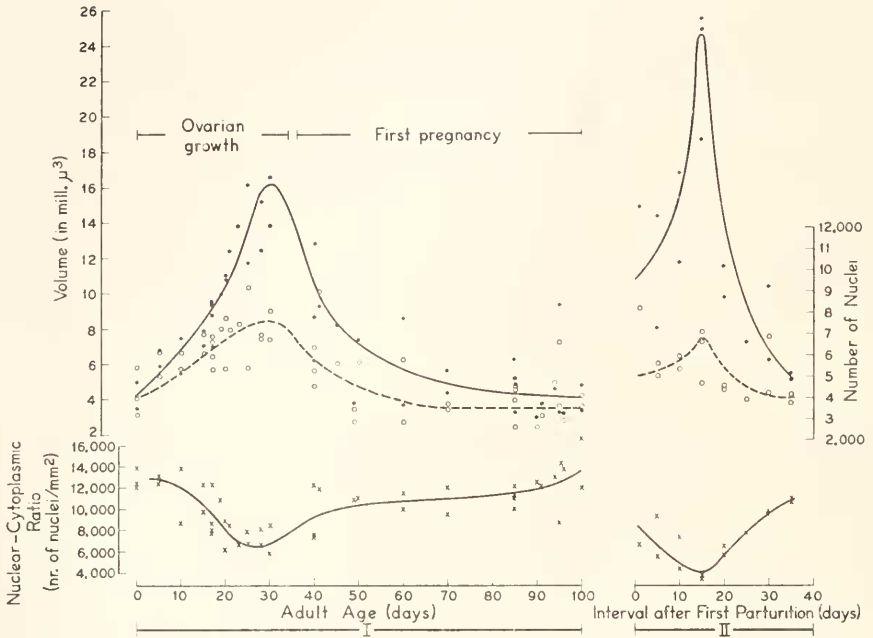


FIGURE 1. Graphic representation of morphological changes in the corpora allata of adult females of *Leucophaea maderae* in conjunction with reproductive cycles. —●—●—●— = volume of both corpora allata in million μ^3 ; ----○----○---- = number of nuclei, calculated for both glands; —×—×— = nuclear-cytoplasmic ratio (number of nuclei/mm.²). Diagram includes (I) entire period of first reproductive cycle (from emergence of adult to first parturition at about 100 days of adult age), (II) second reproductive cycle (from first parturition to early part of second pregnancy). In this and subsequent figures of this series the curves do not represent mathematically correct summaries of the quantitative data which do not lend themselves to such treatment; they are merely intended to facilitate the visualization of the changes over the periods indicated. Note that volumetric changes are paralleled by changes in nuclear numbers and in nuclear-cytoplasmic ratios. For details see text.

as those of newly emerged females. This level was maintained throughout "pregnancy" to be followed by a more pronounced and more rapid rise (maximum volume 25.5 million μ^3) reached after 15 days in the pre-ovulation period of the second cycle. A study of this and older groups (Table I) indicated that essentially the same periodic changes in corpus allatum volume in conjunction with alternating phases of activity and inactivity occur in consecutive reproductive cycles. The first cycle differs from the subsequent ones only in degree in that the volumetric maximum is lower and is reached more slowly. In this respect our data agree with those of Engelmann (1957).

We came to different results, however, with respect to the remarkable cellular changes accompanying the periodic increase and decrease in corpus allatum volume. While it is true that the nuclei are more widely spaced in large, active corpora allata than in small, the rise in glandular volume is not exclusively due to an increase in the amount of cytoplasm. The contribution made by the nuclei in this growth process is primarily by a rise in their number, and less by an increase in

their size. In our series, the total number of nuclei estimated per pair of corpora allata rose from a minimum of 3128 in newly emerged females to a maximum of 9220 in the first, and of 8236 in the second pre-ovulation period.

The highest nuclear count of the whole series (15,591) belongs to a female which also has the highest volumetric value for normal corpora allata (37 million μ^3). This case is noteworthy in that it is a female with the exceptional adult age of 599 days carrying large ova at the time of fixation. Its corpora allata, in addition to the large number of normal sized nuclei, contain a giant nucleus (diameter 46 μ) comparable to those described by DeLerma (1932) and Palm (1947) in *Gryllotalpa*. The smallest number of nuclei (2782) occurred in small corpora allata of a 90-day-old female fixed before the onset of the second cycle. This shows the interesting fact that, with decreasing organ volume in pregnant females, the number of nuclei falls to reach again the level characteristic of very young adult specimens.

To sum up, during subsequent periods of activation and quiescence of the corpora allata the increase and decrease in organ volume is accompanied by periodic changes in nuclear numbers. The fact that the volumetric range (3.0–37.0 million μ^3 for both corpora allata) surpasses that of the nuclear numbers (2782–15,591) expresses itself in marked differences in nuclear distribution: Generally speaking, the larger the glands, the fewer the nuclei counted per mm.² (range: 3698–16,765), and the higher the absolute and relative cytoplasmic content of the corpus allatum tissue. The cyclic fluctuations in nuclear numbers are so pronounced that cytological manifestations of these changes should be expected. Signs of mitotic activity, to account for the cell multiplication calculated, have been observed both in our normal and colchicine-treated adult specimens. The number of mitotic figures counted in the corpora allata is altogether small; while they appear to be more frequent during organ growth, mitoses are not entirely restricted to this period.

Conversely, signs of nuclear destruction (pynosis) seem to be more pronounced in corpora allata returning to the inactive state, but are also occasionally found in growing or maximally active glands. This means that growth and regression of the corpora allata are not solely responsible for the fluctuations in nuclear numbers. The shifts in the frequency of mitotic and pynotic nuclei can perhaps be better understood in conjunction with the cytological manifestations of the secretory activity of these glands.

While no comprehensive analysis of the elaboration of the secretory product by the corpora allata of *Leucophaea* is intended in this paper, certain statements may be made. Methods such as Gomori's chrome hematoxylin phloxine, Foot's modification of Masson's trichrome, or hematoxylin and eosin stain are not suitable for the demonstration of secretory materials in these glands. With the aldehyde fuchsin technique, distinct small granules can be demonstrated within the cytoplasm which stain from a rather deep purple to lavender. On occasion larger green staining droplets are observed. Newly emerged animals in our material do not show these granules; in older specimens their number and distribution appear to depend on the functional state.

During what seems to be a rather short and early phase in the secretory cycle certain corpus allatum cells stand out because their cytoplasm is densely packed

with stainable granules. Their presence permits the tracing of cellular processes which are the longer the more central the location of the cell within the gland. In other words, the corpus allatum cells in their mature form are stellate, and obviously release their secretory products by means of processes which end perpendicularly to the surface of the organ. This stellate cell shape cannot be readily ascertained in the absence of secretory granules, since cell boundaries are not always easily observed in the corpora allata. Many specimens show a more widespread distribution of granules which in sections can no longer be brought in spatial relationship with specific gland cells.

In cells presumably representing later stages in the secretory cycle the cytoplasm, instead of being homogeneous, assumes a more or less "stringy" appearance interspersed with vacuoles. These strands of cytoplasm form a characteristic pattern as though applied by strokes of a brush in a direction from the center to the periphery of the gland. It is along these "lines of flow" (Mendes, 1948; Özbas, 1957) that the secretory granules are now oriented, and their direction corresponds to that of the cell processes mentioned above. The fact that the granules tend to become lined up in greater numbers in the periphery of the corpora allata also speaks for their eventual release into the surrounding body fluid.

A further point of interest is that the nuclei of cells containing many secretory granules often appear pycnotic. These and additional pycnotic nuclei, not surrounded by secretory granules, range from slightly shrunken structures to homogeneous intensely staining bodies. Thus, there appears to be in adult corpora allata a continuous cellular turnover, whereby cells becoming exhausted in the process of their secretory function are replaced by the mitotic activity of younger probably non-secreting cell elements. The rate of cellular turnover seems to fluctuate in the course of a reproductive cycle, with the result that activation of the corpora allata is accompanied by increase, and return to inactivity by a decrease in cell numbers.

A further question concerns possible fluctuations in nuclear diameters in relation to periodic changes in organ volume. Measurements of representative cases have shown the nuclear diameter to vary only moderately in normal specimens (from 6.4μ to 8.2μ ; mean 7.0μ). The nuclei of any given specimen may fall within this range, and no definite relationship between organ volume and average nuclear size could be established. The measurements given do not include those of rarely found giant nuclei which seem to occur characteristically in old specimens (see above).

Of all the periodic changes in the appearance of the corpus allatum of *Leucophaea* the one most readily observed is that of the nuclear-cytoplasmic ratio. Thus, even without quantitative determinations, the large, *i.e.*, active corpus allatum can be easily distinguished from the inactive on the basis of its histological appearance.

b. Males

The 46 male specimens studied range from an adult age of 0 to 471 days (Fig. 2). As in the female series, the corpora allata of animals of the same age may show a certain variability in size and nuclear distribution. The smaller average body size of males is reflected in lower corpus allatum values. Shortly after

emergence the glands begin to grow, but this period is shorter than in females. After about 10 days a peak is reached which, on the average, amounts to a volumetric increase of $2\frac{1}{2}$ times over the initial volume. After this, the values level off to fluctuate only mildly throughout the adult stage. The volume of the largest pair of glands measured in this series (9.0 million μ^3) is about four times that of the smallest (2.1 million μ^3). As one might expect, in analogy to the situation in females, the variability in total nuclear counts is somewhat lower in degree than that in volume, but it is nevertheless considerable (range: 2032 to 7000).

Thus, if arranged in the order of increasing volume, male corpora allata also show a gradual increase in the relative amount of cytoplasm. The number of nuclei per mm^2 ranges from 7696 to 17,131. Inasmuch as male corpora allata are on the average smaller than female, their nuclei are generally more crowded. The highest density occurred in a case (adult age: 51 days) which also had the largest absolute number of nuclei; the corpora allata were of medium size. The loosest arrangement of nuclei was observed in an old specimen (adult age: 396 days) with large corpora allata (7.4 million μ^3). The relative cytoplasmic content was almost as high in a male (adult age: 10 days) which had the largest corpora allata in the entire series.

Figure 2 shows quite clearly that the rise in corpus allatum volume during the first ten days of adult life is accompanied by an increase in nuclear numbers and a decrease in the number of nuclei per unit of tissue. The reverse trend occurs after the peak, *i.e.*, in males older than 10 days, but as a group these males do not entirely return to the situation characteristic of newly emerged animals. In principle, the male corpora allata show the same relationships between organ size, number of nuclei, and density of nuclear arrangement as the females, but this relationship is not so pronounced. It is interesting, for example, that within a medium size range (4–5 million μ^3) which encompasses the majority of males

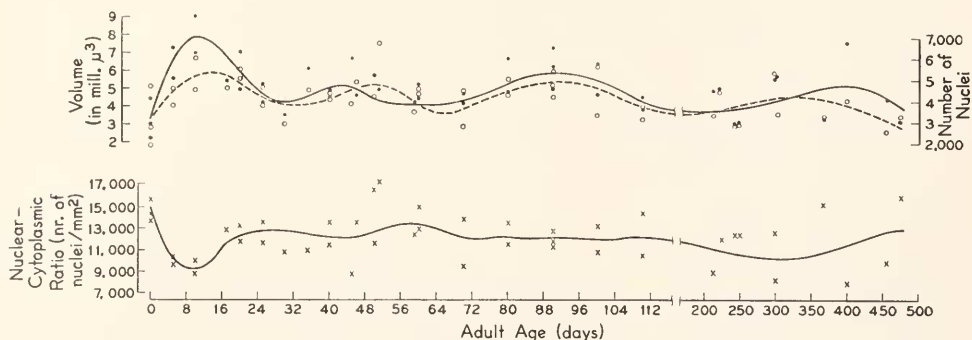


FIGURE 2. Diagram showing morphological characteristics of corpora allata of males of *Leucophaca maderac* ranging in adult age from 0 to 471 days. After a small initial rise (maximum at 10 days) the volumes (—●—●—) level off to fluctuate around a mean value maintained throughout the adult life span. A lack of distinct cyclic activity is also apparent from the values expressing nuclear numbers (----○----○----) and nuclear-cytoplasmic ratios (—×—×—).

studied, the nuclear-cytoplasmic ratio shows the highest degree of variability as compared to that in groups with smaller or larger glands.

The data mentioned so far do not provide evidence for the existence of cyclic activity changes in the corpora allata of adult males. This also applies to the cytology of secretory processes. Purple-staining granules in the cytoplasm have been found in varying amounts in animals of different ages with the exception of newly emerged specimens. While indicating the existence of a secretory function in male adult corpora allata, these cytological phenomena are less impressive than those in females which they resemble in principle. The fact that signs of secretory cycles as well as volumetric and nuclear changes are less apparent in males is related to the greater range in the response of female corpora allata to varying physiological conditions.

DISCUSSION

In most species of insects studied, extirpation and implantation experiments have established the control of reproductive processes by a hormone of the corpora allata (for review see Scharrer, 1955). There are also numerous indications that, at the height of their "gonadotropic" activity, the corpora allata of females are larger than when they are inactive. This was reported among others by Ito (1918), Wigglesworth (1936), Thomsen (1942), Palm (1947), Mendes (1948) and Kaiser (1949). However, most of these and more recent studies (Müssbichler, 1952; Nayar, 1956; DeWilde, 1954; Brandenburg, 1956; Lukoschus, 1956; Lhoste, 1957) do not extend beyond a relatively short interval between emergence and oviposition. A more detailed analysis of this relationship was carried out by Engelmann (1957) who correlated corpus allatum volume and structure with ovarian activity in *Leucophaea maderae*, the species which was also used in the present study. During the long life span of this "ovo-viviparous" species, periods of ovarian quiescence during "pregnancy" alternate with those of activity in which the terminal oocytes grow and deposit yolk, and the accessory sex glands produce secretory material. Only this active phase in the reproductive cycle requires the presence of the corpus allatum hormone while the maturation of the embryos proceeds without it (Scharrer, 1946b). When Engelmann found large corpora allata with relatively high cytoplasmic content in females approaching ovulation, and small glands with densely packed nuclei during pregnancy, he concluded that "activation" of the corpora allata is characterized by an increase in the amount of cytoplasm while the number of nuclei remains constant. The present study, based on a larger material, confirms these earlier results as far as the periodic changes in corpus allatum volume are concerned; it also offers evidence that these changes continue beyond the period (135 days after emergence) analyzed in Engelmann's study, *i.e.*, throughout the adult (reproductive) phase of the insect. In addition, our results demonstrate a participation of the nuclei in the activity cycles of the corpora allata. The role of the nuclei could be ascertained only by quantitative methods in a sufficiently large material, since cell divisions are not observed frequently enough in normal adult specimens. Nuclear counts have shown that, in the first as well as subsequent reproductive cycles, an up to four-fold increase in the number of nuclei may take place during the phase of growth and activation of the corpora allata. The decrease in organ volume which follows is accompanied by a corresponding decrease in nuclear number. These differences in total nuclear

counts are so large that they allow a considerable margin of error which cannot be avoided in the calculation of these figures.

While our cytological observations do not lend themselves to a quantitative evaluation of nuclear changes during the activity cycles of the corpora allata, it is significant that signs of mitotic activity are more prominent in growing glands and pycnotic nuclei are more conspicuous in corpora allata returning to the inactive state. These observations in correlation with the study of the cytology of the secretory cycle lead to the conclusion that corpus allatum cells are used up and replaced during the adult life of the animal. Cyclic changes in the rate of this cellular turnover which accompany secretory cycles in these glands account for the periodic increase and decrease in nuclear numbers and accompanying fluctuations in organ volume.

By comparison, nuclear size showed less variability in our material, so that the main contribution of the nuclei toward increase in organ volume is due to a rise in their number. Taken as a whole, the participation of the nuclear component stays behind that of the cytoplasm which shows not only pronounced quantitative changes but undergoes qualitative transformations during the process of elaboration of secretory granules.

The use of the aldehyde fuchsin technique in the present study for the first time permitted the demonstration of secretion granules in the corpora allata of *Leucophaea*. Although details of the whole secretory cycle still need to be worked out, present evidence supports the view that the active principle is elaborated in the cytoplasm of stellate cells and released into the body fluid surrounding the surface of the gland. Depending on the more peripheral or central location of a cell, its processes may be short or long. They account for the characteristic structural pattern of the gland during certain stages of the cycle in which strands of cytoplasm directed toward the periphery and interspersed with vacuoles are delineated by rows of secretory granules which become more numerous in the periphery of the gland. It is uncertain whether a gland cell remains active only during one secretory cycle or not, but the frequent observations of more or less pycnotic nuclei in cells filled with the secretory product indicate that the cells may become exhausted and are replaced by cells resulting from mitotic divisions in the adult gland. The observation of cytoplasm becoming vacuolized during phases of activity as well as the arrangement of secretory granules along "lines of flow" (determined by the peripheral direction of the cell processes) is in agreement with the findings of other authors in different species of insects (Mendes, 1948; Özbas, 1957).

Thus, in the cyclic activity of the corpora allata of adult females of *Leucophaea* a number of factors are involved. The question arises which of the changes observed express the physiological activation of the corpus allatum. This question cannot be fully answered as yet, but certain known data are of interest in this connection. Increase in nuclear number without relative cytoplasmic increase can be achieved in adult female glands by the implantation of prothoracic glands (Engelmann, personal communication). The absence of ovarian stimulation in animals thus treated indicates that in the corpus allatum of adult *Leucophaea* the relative cytoplasmic increase is an important prerequisite for its activation. The possibility that it is the only one is illustrated by the situation in those species where corpus allatum growth in adults is said to take place solely by an increase in cell volume and

nuclear volume, but not in cell number (Brandenburg, 1956; Lukoschus, 1956). It is evident from Engelmann's data as well as our own (Fig. 1) that at the first peak of activity the corpus allatum volume does not reach the same level as at the second and subsequent ones. Conversely, more time is required for the stimulation of the ovary during the first reproductive cycle than later on. It seems that the corpus allatum may require a short period after emergence to complete its adult development, as has been postulated also for other organs (Rockstein, 1956). The gland may, therefore, not be capable of complete "activation" until after the first cycle.

The possibility that such post-emergence maturation takes place is also indicated by the moderate rise in volume and nuclear numbers occurring in male corpora allata within the first ten days of adult life. Our own observations in this respect are in line with those of Engelmann (personal communication) in *Leucophaea* and by Mendes (1948) in *Melanoplus*. At any rate, no interpretation other than one of tissue maturation can be given for the initial volumetric increase of the corpora allata of adult males, as long as their functional role is so little understood. All we know is that the male reproductive activity in *Leucophaea* is undisturbed after allatectomy (Scharrer, 1946b), and that certain data suggest a relationship between corpora allata and metabolic processes (Samuels, 1956). A sustained control of metabolic functions by the corpora allata might well account for the picture of "mild activity" frequently observed in histological preparations of adult male glands of every age. This steady appearance, which is in contrast to the cyclic pattern in the corpora allata of adult females, concerns range in organ volume and nuclear number as well as cytological manifestations of secretory activity.

In connection with the observation in both sexes of corpus allatum stimulation following emergence it is of interest that this early adult period in *Leucophaea* also differs from later ones with respect to certain metabolic data, such as the animal's lipid content (Scharrer and Wilson, unpublished data).

The present study illustrates that the histophysiological approach whose value is well recognized in vertebrate endocrinology is equally fruitful in the exploration of endocrine mechanisms in insects. In the special case of the corpora allata of *Leucophaea*, known variations of their activity are paralleled by marked changes in the volume of the entire organ, the number of cells, the nuclear-cytoplasmic ratio, and the cytology of the secretory process. The subsequent papers will be concerned with corpus allatum structure following experimentally induced changes in the normal pattern of activity and quiescence of this gland.

SUMMARY

1. The corpora allata of *Leucophaea maderae* display a remarkable degree of structural variability in conjunction with changing functional states. This is particularly apparent in adult females, where a regular sequence of activity and inactivity of these glands parallels alternating phases of ovarian development and quiescence.

2. The volume of active corpora allata surpasses that of inactive glands beyond the range of individual variation, which is considerable. The volumetric rise signalling activation is accomplished to a large extent by an absolute and relative

increase in cytoplasmic content which results in a characteristic "loose" distribution of the nuclei.

3. The present study shows further that the nuclei participate significantly in the cyclic changes of these organs. During each growth period, the nuclear numbers increase up to several times the original values. When, after ovulation, the corpus allatum returns to a state of inactivity which is maintained during pregnancy, the nuclear-cytoplasmic ratio returns to a level characteristic of the newly emerged female. The accompanying reduction in cell number to the initial level is evident not only from a drop in nuclear counts but from the observation of pycnotic nuclei.

4. The differences in the frequencies with which nuclear pycnosis on one hand, and mitotic figures on the other are observed in various stages suggest the existence of a cellular turnover which seems continuous but whose rate changes periodically. During activation of the corpus allatum, when secretory products are elaborated, the increase in cell number surpasses the rate of cell destruction. In the regressing gland the latter process predominates over that of cell replacement.

5. With the use of a modified aldehyde fuchsin technique distinct secretory granules have been demonstrated in the corpus allatum cells of *Leucophaea*. The granules line up along processes of the cells which are directed to the surface of the corpus allatum. This fact, as well as the accumulation of stainable granules in the periphery of the gland, speaks for the release of the active substance into the surrounding hemolymph. The occurrence of pycnotic nuclei in cells filled with secretion granules suggests that these gland cells may become exhausted fairly quickly, perhaps in the course of one secretory cycle.

6. By comparison with the situation in the females, the corpora allata of adult males show considerably less variability. Soon after emergence, a short period of "activation" seems to occur, as judged by the same structural characteristics as in the females. After that a fairly constant level of presumably mild activity appears to be maintained throughout adult life. Since the available information on the functional role of the corpora allata in male adult animals suggests no pattern of periodicity, the lack of distinct cyclic changes in the morphology of these glands is not surprising.

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